AD-A256 392	**PAGE**	Form Approved OBM No. 0704-0188

Public reporting t... onse, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the d... ...ments regarding this burden or any other aspect of this collection of information, including suggestions for reducing this l... ...rations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Man... ..., DC 20503.

1. Agency U	1992	3. Report Type and Dates Covered. Final - Journal Article

4. Title and Subtitle.
Computerized oceanic particle characterization using heavy metal staining, SEM, EDXS and image analysis

5. Funding Numbers.
Contract

Program Element No.	0603704N
Project No.	00118
Task No.	300
Accession No.	DN255048
Work Unit No.	13318P00

6. Author(s).
Dennis M. Lavoie

7. Performing Organization Name(s) and Address(es).
Naval Oceanographic and Atmospheric Research Laboratory
Ocean Science Directorate
Stennis Space Center, MS 39529-5004

8. Performing Organization Report Number.
JA 333:057:90

9. Sponsoring/Monitoring Agency Name(s) and Address(es).
Naval Oceanographic and Atmospheric Research Laboratory
Ocean Science Directorate
Stennis Space Center, MS 39529-5004

10. Sponsoring/Monitoring Agency Report Number.
JA 333:057:90

DTIC
ELECTE
S OCT 0 9 1992
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11. Supplementary Notes.
Published in Deep-Sea Research

12a. Distribution/Availability Statement.
Approved for public release; distribution is unlimited.

92-26835

13. Abstract *(Maximum 200 words).*

Heterogeneous particles collected from seawater were analysed for physical dimensions and elemental composition using a computer-controlled image and chemical analysis system based on a scanning electron microscope and energy dispersive X-ray spectrometer. Staining the particles with the heavy metal ruthenium largely overcame the difficulties of forming an adequate image of organic particles that usually limits such automated analyses. Ruthenium staining provides an image comparable to that achieved with metal coatings, improves the contrast of organic particles, and provides a more uniform background grey level. In additon, this treatment simplifies sample preparation and improves the X-ray count rate compared with metal-coated specimens. The major X-ray energy peak of ruthenium interfers only with that of chlorine, which is usually removed during the sample preparation procedures.

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92 10 8 069

14. Subject Terms.
Optical oceanography, optical instrumentation, physical oceanography

15. Number of Pages.
14

16. Price Code.

17. Security Classification of Report. Unclassified	18. Security Classification of This Page. Unclassified	19. Security Classification of Abstract. Unclassified	20. Limitation of Abstract. SAR

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

INSTRUMENTS AND METHODS

Computerized oceanic particle characterization using heavy metal staining, SEM, EDXS and image analysis

DENNIS M. LAVOIE*

Abstract—Heterogeneous particles collected from seawater were analysed for physical dimensions and elemental composition using a computer-controlled image and chemical analysis system based on a scanning electron microscope and energy dispersive X-ray spectrometer. Staining the particles with the heavy metal ruthenium largely overcame the difficulties of forming an adequate image of organic particles that usually limits such automated analyses. Ruthenium staining provides an image comparable to that achieved with metal coatings, improves the contrast of organic particles, and provides a more uniform background grey level. In addition, this treatment simplifies sample preparation and improves the X-ray count rate compared with metal-coated specimens. The major X-ray energy peak of ruthenium interferes only with that of chlorine, which is usually removed during the sample preparation procedures.

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1. INTRODUCTION

THE scanning electron microscope (SEM), in combination with an energy dispersive X-ray spectrometer (EDXS), can be a powerful tool for counting, measuring and chemically analysing particulate material collected from oceanic or fresh waters. In initial applications (BASSIN, 1975; JEDWAB, 1980; DEHAIRS, 1980; LAMBERT *et al.*, 1981), the operator located individual particles, measured size and shape, did the EDXS analysis, then compiled all the data, a rather labor intensive procedure for routine studies. Several laboratories developed computer-automated techniques for analysis of various feature types (e.g. KELLY *et al.*, 1980; HANNA *et al.*, 1980; also see LEE and KELLY, 1980). Using a system developed by E. W. White and colleagues, called CESEMI-2, BISHOP and BISCAYE (1982) analysed thousands of oceanic inorganic particles during the GEOSECS program, demonstrating the potency of the technique for developing statistically significant,

*Oceanographic Division, Naval Oceanographic and Atmospheric Research Laboratory, Stennis Space Center, MS 39529, U.S.A.

multidimensional data on particle populations in the water column. Employing a different system, Yin and Johnson (1984) and Weidemann and Bannister (1985) analysed suspended particles in lakes, and Carder et al. (1986) studied aeolian Saharan dust collected in aerosol samplers and in a sediment trap deployed in the Sargasso Sea. These marine applications of the technique were generally applied to the analysis of inorganic particles only, partly because of the difficulties in imaging organic particles with contrast that is sufficient for an automatic analysis system. This paper describes a simple technique that enables both organic and inorganic particles to be detected and analysed by such a system, thereby increasing its usefulness for marine studies. The technique was used on particulate samples collected from the Sargasso Sea, and several treatments of the data are presented to illustrate the possibilities.

SAX operating principle

The combination of AIA/SEM/EDXS was termed SAX (Scanning electron microscopy with Automated image analysis and X-ray energy spectroscopy) by Johnson (1983), and, in the interest of brevity, this acronym will be used here. Lee and Kelly (1980), and Johnson (1983) provide good overviews of the operating principles of SAX, which will only be covered briefly here. The SAX concept was commercialized by LeMont Scientific, Inc. (State College, PA), and this report is based on the use of their instrument as applied to particles.

SAX is a feature-oriented, real-time technique (Lee and Kelly, 1980). It is analytical in that it extracts quantitative information·about observed, individual features (Jones and Smith, 1978; Johnson, 1983). In contrast to image analysis techniques that operate on a stored image (usually a photograph or a digital image file), SAX operates in an "active" image acquisition mode: all analyses are performed and recorded while the sample is under the SEM electron beam. Although this approach is slow, relative to modern image frame analysers, it enables the elemental analysis to be unambiguously associated with individual particles. In addition, the elemental analysis is more quantitative than can be done using an atomic weight to gray scale correlation, which is the usual approach taken when a frame analyser is applied to this problem.

In setting up the SAX analysis, the operator first sets a threshold level on the image analyser for the SEM signal such that any signal level below threshold is "on background" and any signal level above threshold is "on particle". Under computer control the electron beam is digitally stepped through the field of view in a raster pattern until the signal exceeds the threshold (i.e. until the beam is "on particle"). The digital beam control then guides the beam through present diagonal and grid patterns that determine the physical dimensions of the particle. The resolution of the raster pattern is dynamically adjusted to suit the size of the particle, up to 4096 by 4096 points to allow small particles to be accurately measured even at low magnification, yielding a dynamic size range of about 1:500 (the size of the electron beam is approx. 0.6 nm in cross-sectional diameter in a typical analysis).

The primary physical measurements made are area, perimeter, length and width. From these, various shape descriptors may be derived, along with the coordinates of the geometric center. The computer then directs the electron beam to the center of the particle (or to various proportions of the total particle area), and activates the EDXS for a few seconds to obtain an X-ray energy spectrum. Net X-ray energy counts in pre-designated

energy regions that correspond to elements of interest are storeᴅ along with X-ray count statistics, physical measurements, coordinates and any derived parameters, and the system moves on to the the next particle in the field until all particles have been analysed. The final list of characteristics for each particle is termed an "object vector", and this may be treated as an entity for statistical analysis and classification purposes (KARCICH *et al.*, 1981; JoHᴎSON, 1983). Thus, the technique produces a particle-by-particle description consisting of 30 or more measurements (depending on the number of elements specified), providing a rich data matrix for classification. One mode of classification is by composition. By setting criteria based on the ratios of elemental counts detected in the particle, the system can reliably discriminate among various minerals based on a few seconds of EDXS counts.

The problem of imaging organic particles

The key operation in the automated analysis is the production of an image, for which the preparation of the sample and the set-up of the SEM operating parameters are crucial. What constitutes a good image for the operator, who can easily integrate the visual display to recognize features, is not necessarily a good image for the automated system, which can only deal with signal levels. Setting the threshold is a simple form of image processing that simplifies and speeds the analysis by reducing the informationally complex image to a binary one. As illustrated in Fig. 1, producing a binary image that preserves the morphology of the particles in the microscope field depends on adequate and uniform contrast of the particle relative to the substrate. If the signal level of the particle is not
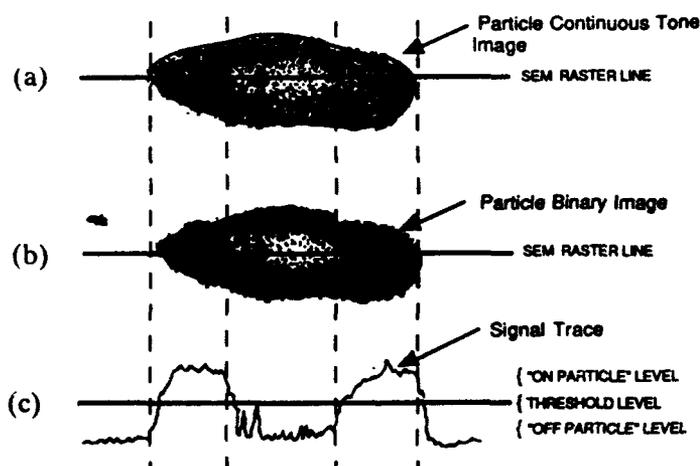
Fig. 1. Relation of SEM signal level to binary image. This figure shows schematically the problem of setting the threshold level for the image analysis when low contrast in a portion of the particle results in a binary image that is not faithful to the original image. (a) The continuous tone SEM image shown with a single raster line (the path of the electron beam across the particle). (b) The binary image. (c) The signal trace observed on the signal oscilloscope with the threshold level superimposed. The light area in the center of the binary image is a portion of the particle which is low in contrast in the continuous tone image, but in the binary image it has the same value as the background and appears to be a hole. This is seen also in the signal trace: the signal at the center of the particle falls below the threshold level and so is not included in the measurement of the feature.

higher than that of the substrate, it falls in the "background" half of the binary image, and the system will not detect the particle; similarly, if this occurs within a portion of the particle, then that portion will be seen by the automated system as part of the substrate or as a void, and it will not be included in the particle measurement. While the human operator can recognize the feature as a whole, discrete particle, the simple automatic system may not.

In the SEM, organic specimens are especially prone to exhibit poor or variable contrast along with "charging" effects (an apparent brightening of edges and points) because of their low atomic weight. To control this problem, SEM microscopists usually coat organic specimens with a metal such as gold or a gold alloy. In order to improve the resolution of surface detail, the secondary electron (SE) signal, and sometimes a reduced electron beam accelerating voltage, are used to restrict the detected electrons to those emitted close to the surface of the specimen. (A discussion of these strategies may be found in Robinson and George, 1978.) Unfortunately, heavy metal coatings and low accelerating voltages decrease the X-ray emission rate, necessitating longer count times to maintain the sensitivity and accuracy of the elemental analysis. Thus, the SAX analyst is apparently caught on the horns of a dilemma, between obtaining a usable image and reasonable X-ray acquisition times.

Heavy metal staining

It is important to note that the production of a binary image for automated analysis does not require good surface resolution, only an image whose outline is faithful to the particle morphology and a signal level from the particle that is relatively constant and consistently higher than that of the substrate. One way to accomplish this is to increase the effective atomic number of the organic particles by impregnating them with a heavy metal. Heavy metal "stains" are commonly used in transmission electron microscopy to render biological tissue more electron dense. Osmium tetroxide (OsO_4) is commonly used because its volatility makes for convenient sample preparation by exposure to its vapor. Unfortunately for EDXS, the osmium M-alpha peak is at 1910 eV. With a FWHM (full width at half maximum) of some 80 eV, it interferes with the silicon L-alpha peak at 1740 eV and the phosphorus L-alpha peak at 2015 eV. Both these elements may be of interest in suspended particle studies.

Ruthenium tetroxide (RuO_4) is a less frequently used but more suitable heavy metal compound. It is a stronger oxidizer, and it reacts with a variety of organic compounds (Hayat, 1989). For EDXS, the ruthenium L-alpha peak at 2558 eV interferes seriously only with the chlorine K-alpha peak at 2622 eV. In studies of marine suspended particles, chlorine is removed during the desalting and dehydration process, so its place in the EDXS spectrum is unoccupied.

When a polycarbonate-type filter membrane and the particles captured on it are both stained with ruthenium, there are two other salutary effects. The first is that the substrate and particles alike are rendered conductive throughout their volume, eliminating the requirement for surface coatings to control charging. The second effect is that the signal from the filter substrate is uniformly low, while that from the particles is high. This is due to the volume backscatter effect (Robinson and George, 1978): even when the sample is heavily stained with ruthenium, the concentration of the metal in the particles and filter is still low enough that the electrons in the beam penetrate deeply and are thus backscattered

throughout a relatively large volume. In a flat and uniform polycarbonate membrane filter, such as a Nuclepore™ or Poretics™, most of the backscattered electrons (BSE) are reabsorbed within the body of the filter, leaving relatively few to escape to be detected. The result is a low and uniform signal level for the filter substrate; however, a particle sitting on top of the filter has BSE escaping from most of its surface, and as a result is much "brighter". (To the extent that the BSE also cause the secondary electron (SE) signal due to their absorption by the sample, the volume backscatter effect also improves the contrast of the SE image.)

The uniformity of the signal detected from the particle is enhanced by using a quad BSE detector, i.e. four detectors attached to the pole piece directly above the sample. To eliminate topographical effects and to increase the sensitivity, the signals frc.ii the quad detector are summed. At magnifications ≤500×, the BSE signal provides more uniform contrast than the SE signal and minimizes charging (ROBINSON and GEORGE, 1978). For small particles at magnifications ≤100×, however, there is little practical difference between the two types of signal.

2. PROCEDURE

To test the technique on actual oceanic particles, seawater samples were collected during Cruise EN166 on the R.V. *Endeavor* in August 1987 in the vicinity of 34°N, 70°W.

Sample collection

Seawater was collected using 10-l Niskin bottles mounted on a rosette sampling device (General Oceanics, Inc., Miami, FL). The rosette, which also was equipped with a CTD, fluorometer, and transmissometer, was sent depth to establish the temperature, salinity, transmissometry, and fluorometry profiles, and the bottles were tripped as the rosette was paused at discrete depths during retrieval. Subsamples of the Niskin bottle contents were drained into clean, glass, 1-l reagent bottles. Aliquots of 250 ml were passed through 25 mm diameter, 0.4 μm pore size (Nuclepore Corp, Pleasanton, CA) or Poretics (Poretics, Corp., Livermore, CA) polycarbonate membranes to collect the particles. Filtration with a vacuum of about 1 Pa (1 atm) was done in glass filter funnels capped with aluminum foil to prevent airborne particle contamination. The filters were then laid, particle side up, on cellulose pads saturated with EM grade glutaraldehyde (2% in filtered seawater) for fixation and storage at 4°C in tightly sealed, disposable culture dishes (Millipore Corp, Bedford, MA) until they were processed for SEM examination.

Sample processing

In the laboratory, enough 0.5% RuO_4 solution was put in a Petri dish to completely cover the bottom (approx. 5 ml). A piece of filter paper dampened with distilled water was placed on the inside of the cover, and the polycarbonate membrane with the sample side up was laid on top of it. The cover was then inverted and put onto its container. The purpose of the damp filter paper was to hold everything in place by surface tension, and to prevent the sample from drying out during the RuO_4 treatment. The sample was then exposed to the RuO_4 vapor for 15–30 min, at which point it was a uniform charcoal gray

color. It was then removed, desalinated, rinsed, and dehydrated using the saturated pad technique of DRIER and THURSTON (1978), and then critical point dried by the standard techniques (HAYAT, 1989).

Safety precautions

RuO_4 is available as a ready to use 0.5% aqueous solution from Polysciences, Inc., 400 Valley Rd., Warrington, PA. Data sheet No. 320 should be requested when the solution is ordered for safety and handling precautions.

RuO_4 solution is toxic, and it is a strong oxidizer that reacts vigorously when the solution contacts most organic compounds, so it should contact only glass. It should be used with the same personal safety precautions as OsO_4, including fume hood, flowing water nearby, goggles, and long sleeves. Latex surgeons' gloves provide good protection and good dexterity (it is also instructive to watch the light-colored latex darken on brief exposure to the RuO_4 vapor).

The solution is a clear yellow, but it is readily decomposed to a colorless solution by ambient light at room temperatures so, after the sealed ampule is opened, the solution should be stored in a foil-wrapped, air-tight container and kept in a refrigerator. The small volume used for the vapor treatment can be reused if it is returned to a storage bottle as soon as it is no longer needed.

Sample analysis

Image and chemical analysis was performed using a LeMont Scientific (State College, PA) model DA-10 which controlled an AMRAY (Bedford, MA) model 1000A SEM and a KEVEX (Foster City, CA) model 7000 EDXS. The BSE signal was detected with a LeMont Scientific quad BSE detector. Magnification was $50 \times$ and the minimum size particle for analysis was set to 1 μm. The system was set to analyse all particles at full resolution (4096×4096), and X-ray collection time was set to 2 s live time, which was sufficient time to differentiate elements by the ratioing technique. With these operating parameters, the system required approx. 6 s of real time per particle and analysed 400–700 particles per hour.

Energy intervals ("regions of interest" or ROIs) of approx. 140 eV were set on the X-ray spectrometer, centered on the characteristic peak energies of each of 25 elements, including ruthenium, and appropriate corrections were specified for each ROI. After the X-ray energy spectrum was collected for an individual particle, the corrections were applied, and the ruthenium peak was stripped out of the X-ray energy spectrum. Next, the ratios of elements in the sample were computed from the relative number of X-ray counts (intensities) in each region. In the final step, the system classified the particle using the operator-specified scheme, compiled the results, and printed out a report in various tabular and graphical statistical formats. The raw data were automatically saved on disk.

In the classification scheme used here, particles whose X-ray counts occurred exclusively in the region of the ruthenium peak yielded a net spectrum with no peaks above background, and these were classified as organic (beryllium-window X-ray detectors cannot detect elements below sodium in the periodic table, e.g. carbon, oxygen and nitrogen); those exhibiting >95% of their X-ray counts as silicon or calcium were classified as silicon-rich or $CaCO_3$, respectively; siliceous particles with various proportions of

Table 1.   *Classification scheme* for suspended marine particulates (after D. L. JOHNSON, personal communication)*

Class name	Criteria	Major specifications
Organic†	Low or no X-rays	Total net X-ray count <70
Silicon-rich	Si counts predominate‡	Si = 95–100% of total X-ray counts
Silicates	High Si plus varying proportions of other elements	Si = 25–100% of total X-ray counts and Al, Mg and/or Ti = 0–50% and/or Na, K and/or Ca = 0–35% and/or Fe = 0–45% and/or S = 0–20% and/or others = 0–5%
BaSO₄	Ba and S occur in prescribed ratio range	Ba/S = 18–560% and (Ba + S)/others >50%
CaCO₃	Ca counts predominate	Ca = 95–100% of total X-ray counts
CaSO₄	Ca and S occur in prescribed ratio range	Ca/S = 30–350% and (Ca + S)/others >65%
Ca–Mg minerals	High Ca and Mg plus varying proportions of other elements	Ca = 35–100% and Mg and/or P = 0–100% and/or Si = 0–25% and/or Fe, Mn and/or S = 0–20% and/or others = 0–10%
Ca–Si minerals	Ca and Si occur in prescribed ratio range	Ca/Si = 50–200% and (Ca + Si)/others >65%
SrSO₄	Sr and S occurring prescribed ratio range	Sr/S = 50–200% and (Sr + S) others >50%
Sulfates and sulfides	High S + others	S fraction = 20–100% and Si = 0–80% and/or Cd, Fe, Mn, Pb, S and/or Zn = 0–80% and/or others = 0–15%
Metallics	Metallic elements predominate	Al, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Pb, Hg and/or Cd = 10–100%
Miscellaneous	Meet no other criteria	None

*The classification is linear, i.e. for each particle, the table is scanned from the beginning, and the particle is put into the first class for which it satisfies the specifications.

†The ruthenium peak is subtracted from the spectrum before relative peak intensities are determined. Particles whose net spectrum consists of only background counts are assumed to be organic.

‡Fractions and ratios of elements are determined (after appropriate corrections are applied) based on the relative intensities (counts) in each peak.

sodium, magnesium, aluminum, iron and potassium were classified as silicates; and so on. An outline of the classification scheme is given in Table 1. The LeMont software allows the specifications for the various classes to be set in a variety of ways, e.g. by shape, area, or count rate. This study used elemental proportions and ratios based on relative X-ray peak intensities. This classification scheme is a linear type, i.e. it operates much like a sieve, in that a particle is assigned to the first class in which it satisfies all the specifications, and no other classes are checked for a possible fit. Such a classification scheme requires the careful construction of the specifications for proper operation. JOHNSON (1983) discusses the pros and cons of such schemes as well as alternatives. The classification used here was based on

ones suggested by D. L. JOHNSON (personal communication, 1988) and was intended to be general enough to cover most particles that might be encountered in the marine environment. In use on Sargasso Sea particulates, this scheme accounted for approx. 98% of the particles analysed.

Data reduction

The relevant physical measurements provided by the system were particle number and particle area (perimeter was also measured, but this parameter was not used here). Particle counts per unit volume of seawater were calculated from the proportion of the total filter area examined and the amount of seawater processed. The particle area measurement may be used directly as the particle size, or the size may be expressed more conventionally as the diameter of a sphere having the same area (the "area equivalent diameter", or AED). Similarly, particle volume may be estimated by computing the volume of a sphere having the AED of the particle or by rotations of specific particle two-dimensional shapes about an axis.

One of the potential applications of SAX data to oceanographic problems is to estimate refractive indices of suspended particles to provide "ground truth" for optical studies. Not only can the relative proportions to the total particle population be determined for each category of refractive particle at each depth, but their size and shape distributions can be specified as well. To illustrate this, a weighted average refractive index relative to seawater, m', was computed for each of the three depths by:

$$m' = \Sigma \ (n_i p_i)/n_W.$$

Table 2.   *Relative refractive indices*

Class	m'	Reference
Organic	1.03	AAS, 1981
Silicon-rich	1.06	McCRONE et al., 1967
Silicate	1.13*	WEAST, 1979
CaCO₃ (calcite)	1.25	WEAST, 1979
Ca/Mg (dolomite)	1.26	WEAST, 1979
CaSO₄ (gypsum)	1.14	WEAST, 1979
BaSO₄ (barite)	1.22	WEAST, 1979
Sulfur and sulfides	1.58†	WEAST, 1979
Metallics	2.37‡	WEAST, 1979

m' = Refractive index relative to seawater = $n/1.33$, at 589.3 nm (sodium line). (Refractive index of seawater = 1.33, NEUMANN and PIERSON, 1966).

*Average m' of montmorillonite (n = 1.48), illite (n = 1.54), kaolinite (n = 1.53), phillipsite (n = 1.48), orthoclase feldspar (n = 1.51).

†Average m' of marcasite (FeS, n = 1.55), oldhamite (CaS, n = 2.14), sphalerite (ZnS, n = 2.37), wortzite (ZnS, n = 2.36).

‡Average m' of hematite (Fe₂O₃, n = 3.22), magnetite (Fe₃O, n = 2.42), goethite [FeO(OH), n = 2.27], lepidocrocite [FeO(OH), n = 1.94], zincite (ZnO, n = 2.01).

where n_i is the literature values of the refractive index, n_i, for each chemical class; p_i is the proportion of the total population for each chemical class; and n_w is the refractive index of seawater.

Table 2 lists the basis for the calculations. Alternatively, the refractive index may be calculated for various subpopulations as well, such as those based on elemental composition, size or shape.

3. RESULTS

Hydrographically, Sta. 11 was typical of the Sargasso Sea stations examined on cruise EN166. The temperature profile (not shown) indicated a mixed layer at 24.5°C to 30 m, then a steady decrease with depth until 72 m, at which the temperature dropped from 21 to 20°C within the space of 5 m. In-situ relative fluorometry was uniformly low until the depth of this temperature step, at which point it increased about 5%. In-situ transmissometry also exhibited a monotonic profile until 72 m, but the increase was <1% at that depth.

Particle data for three depths—10, 30 and 75 m—are presented in Figs 2 and 3. Figure 2 is a composite of three measurements vs four chemical classes, plus an inset table showing total counts and volume for each depth. Figure 3 shows the particle size distribution for particles in the three major chemical classes plus the distribution for all classes considered together.

In bulk terms, the table inset in Fig. 2 indicates that both the total particle count and the total particle volume decrease with depth; however, the breakdown by chemical class (horizontal bars) illuminates other aspects of the data. One is that the organic fraction predominated the total numbers of particles, and that the proportion of each chemical class in the total count remained relatively constant with depth. In terms of particle volume, however, organic particles and silicon-rich particles were similar, except at 10 m, where they differed by a factor of about 5. Both counts and volumes showed a declining trend with depth in three chemical classes, but in the silicon-rich class, there was a slight increase in both parameters at the 75 m depth.

The total particle volume of the silicates and others was a small proportion of the total at all three depths.

Calcium particles were seen only in the 10 m sample. They constituted only about 1% of the total count and about 5% of the total volume at this depth.

Particles that were predominantly metallic or that contained significant sulfur were, taken together, more numerous than silicate particles. The metallic particles were small, all falling between the low diameter cutoff of 0.5 and 8 μm. Although sparse compared with organic or silicon-rich particles, they were present in numbers on the order of 10^6 to 10^7 m^{-3}. These particles were predominantly iron (73% of the total) and calcium (13% of the total). Sulfur-bearing particles were not detected in the 10 m sample but were present at 30 and 75 m at concentrations up to 10^6 m^{-3}. The sizes of these particles were small, between 0.5–6 μm.

Examination of the particle size distributions in Fig. 3 shows that the size–volume distribution of the general particle population is mostly controlled by that of the organic particles, especially at 10 m depth. At the other two depths, all three classes exhibit relatively flat distributions except for one or more peaks at the large end of the distribution.

Because of the dominance of two major chemical classes, the mean relative refractive
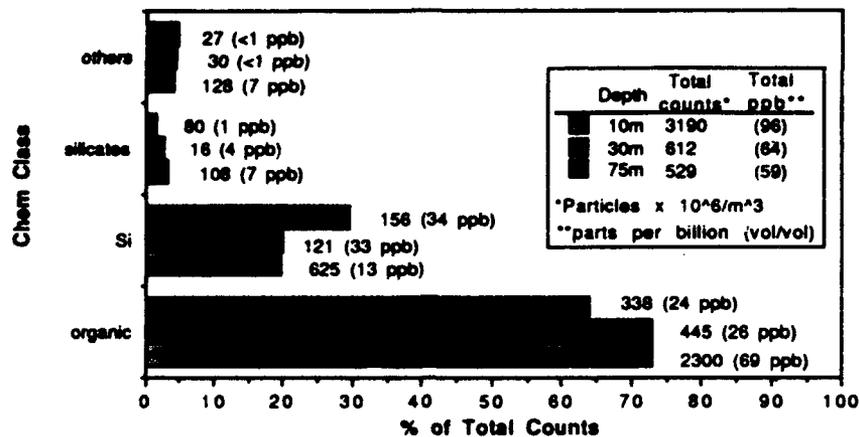
Fig. 2.   Particle statistics of four chemical classes (organic, silicon-rich, silicate minerals, and all others) at 34°N, 70°W (Sargasso Sea) in August 1987. See Table 1 for the classification scheme.
   The values in the inset table indicate the total count and total ppb at each depth. The horizontal bars indicate the percentage of the total number of particles represented by each class at each depth; the values to the right of each bar are the total particle counts in that class and depth and, in parentheses, the parts per billion (volume of particles per volume of seawater) occupied by the particles of that class at each depth.

index based on numerical abundance for the particle population changes only slightly among the three depths, i.e. $m' = 1.08$ at 10 m, 1.07 at 30 m, and 1.09 at 75 m.

4. DISCUSSION

This data set is presented only to illustrate the possibilities inherent in the SAX technique when it is modified to analyse organic as well as inorganic marine particles. Because of the recognized shortcomings in the methodology used at the time, these results should not be taken as a definitive study of the conditions at this station. Some of the shortcomings are due to problems inherent in sampling marine particles (such as the breaking of large, fragile particles, possibly composed of heterogeneous materials, into smaller particles whose average composition may be different from the average composition of the parent), while others are due to the sensitivity of the technique, but all deserve some discussion.

Of particular concern for the SAX technique is the possibility of contamination during collection and preservation. This may occur from such things as the shedding of rubber particles into the sample by the internal spring of the Niskin bottle or of metallic particles from the hydrowire into the surrounding seawater, and the settling of particles from the shipboard laboratory air onto an uncovered filter. Contamination from these sources could be ameliorated by physical measures such as using sterile, bag-type samplers or Niskin "Go-Flo" bottles; using Kevlar-armored hydrowire; by triggering the samplers without pause as the rosette is descending; by using a portable laminar flow, clean air hood in the shipboard laboratory when working with the filters; or, as was done here, to cover samples during vulnerable procedures.

An alternative method for dealing with contamination, especially in difficult conditions,
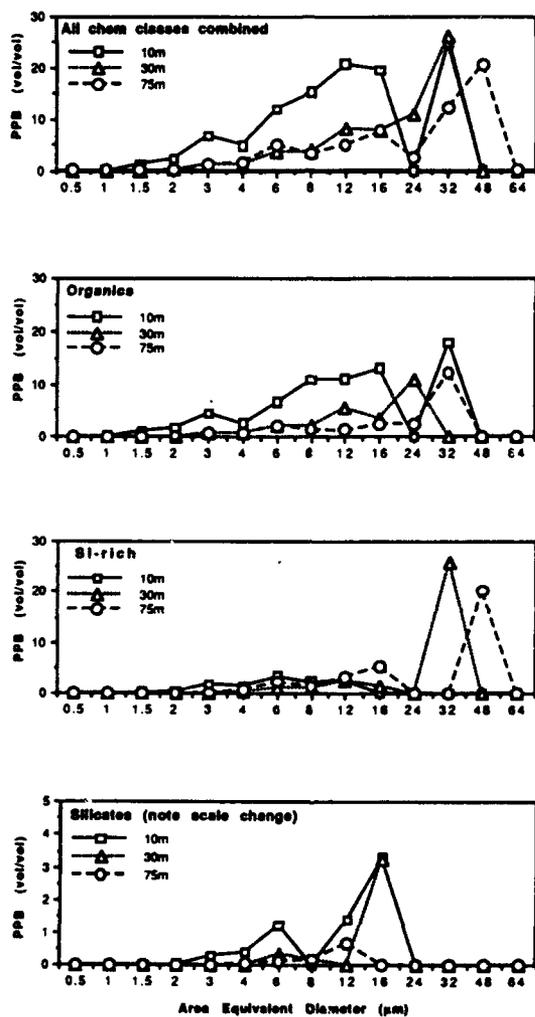
Fig. 3. Particle size distributions by chemical class and depth at 34°N, 70°W (Sargasso Sea) in August 1987. See text for description of the chemical classes. Horizontal axis: particle size as AED in mm (the diameter of a sphere with a projected area equivalent to that of the particle). Scale is $\log_2$. Vertical axis: particle volume as parts per billion (volume of particles per volume of seawater). In this type of presentation, each diameter interval (2^n) contains equal mass if all particles are the same density (SHELDON and PARSONS, 1967; SHELDON et al., 1972).

is to use the classification ability of the SAX system itself. Known or suspected sources of contamination could be sampled and analysed to determine their signature (elemental ratio or some other basis), and that signature then could be assigned to an explicit class to identify contaminant particles at analysis time. Post-cruise EDXS analysis of samples of Niskin springs and used armored hydrowire indicated that these sources could potentially contribute iron, aluminum and sulphur particles to the water.

Although contamination is a possibility in this sample set, there may have been natural sources of metallics as well. Fe_2O_3 as well as Al_2O_3 have been reported to be associated with diatoms (HARVEY, 1937; VINOGRADOV, 1953). Another possible source of metal-bearing particles is the aerolian input of Saharan dust (PROSPERO et al., 1970; CARDER et al., 1986). In the most nearly comparable study (BISHOP and BISCAYE, 1982), however, metal- and sulphur-bearing particles were rare.

Chemical artifacts may be introduced during preservation also. Numerous coccoliths would be expected to be present in this region of the ocean (McINTYRE and BÉ, 1967), but their absence in these samples indicates that acidic preservation conditions dissolved any calcium carbonate particles present.

As mentioned previously, if morphology is important to the analysis, both the preservation and the preparation techniques must be designed toward this end. For example, provided the particle morphology is preserved, the SAX technique can be used to discriminate between amorphous organic particles and free bacteria and other micro-organisms, based on elemental composition and shape. Thus the technique might be used in a study of the sub-micron sized particles that have been reported to be numerous in oceanic waters (KOGURE and KOIKE, 1987). While the saturated pad technique used here is reported to preserve bacterial morphology (DRIER and THURSTON, 1978), its effect on specific particles of interest should be determined.

Keeping in mind the limitations of this data set, the results illustrate the type of information that may be obtained from the application of the SAX technique to the study of marine particulates. Like the resistive pulse ("Coulter Counter") technique, SAX can give bulk population characteristics such as total counts or volumes with depth (inset table in Fig. 2); however, the compilation of several hundred object vectors, each representing an individual particle, allows much more detailed analysis of the particle population in the water column, as suggested by these results. In addition, the data matrix can be examined in several different ways. The particles in Fig. 2 and the size distributions given in Fig. 3 were classified by their chemical make-up, but they could have been classified just as easily by some physical parameter, such as area, diameter, perimeter or shape.

Further work is necessary, comparing the results of the SAX technique to other techniques currently used in oceanography, such as optical microscopy, resistive pulse, and flow cytometry; however, it should be borne in mind that the various methods measure different aspects of marine particles and that each has its own limitations, so the results may not be strictly comparable for a natural, heterogeneous population. For intercomparison studies, the most reliable results would be obtained using defined particle populations.

In the context of intercomparisons, it should be noted that it is possible in SAX to do a post-analysis visual examination of unusual particles found during the analysis or of a particular class of particles. Given some careful forethought to accurate positioning and registration of the sample stub in the SEM, it is feasible to use the particle coordinates that are part of the object vector to return to an individual particle on the sample filter. In

addition, the system can also be interactively restricted to the analysis of a single particle or a limited sub-field of particles.

5. CONCLUSIONS

The power of the SAX methodology is in the multidimensional data that it extracts from the sample. Each particle is unambiguously characterized as to important elemental and physical properties, and the resulting object vectors may be analysed in a variety of ways, depending on the goals of the investigator. The ruthenium impregnation technique described here allows SAX to be applied reliably to oceanic samples consisting of heterogeneous particle populations for such marine studies as: ocean optics, chemical flux, gross ecological structure, aeolian deposition, and fluvial input and transport. Further technical development is feasible, yielding faster throughput and more parameters, including Fourier and fractal shape factors and better volume estimates.

The SAX technique may be particularly useful in providing "ground truth" for optical studies. Not only can the relative proportions to the total particle population be determined for each category of refractive particle at each depth, but their size and shape distributions can be specified as well.

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